TECHNICAL REPORT
FD-24

# RESISTANCE OF MICROORGANISMS TO IONIZING RADIATION APPLIED TO FOODS



by

A. W. Anderson
OREGON STATE UNIVERSITY
Corvallis, Oregon

Contract No. DA 19-129-QM-2035

September 1965

U. S. Army Materiel Command
U. S. ARMY NATICK LABORATORIES
Natick, Massachusetts

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Project Reference:
7-84-01-002

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U. S. ARMY NATICK LABORATORIES
Natick, Massachusetts 01762

## FOREWORD

Relatively high dose levels of ionizing radiation are needed to make foods sterile. These high dosages frequently produce deleterious organoleptic changes in the product. The degree of food spoilage, and the cost of the sterilization process, will vary directly with the quantity of ionizing energy applied. Hence, means must be sought to lower sterilizing doses.

This contract undertook a study on the synergistic lethal effects of food additives and radiation on spores of Clostridium botulinum in meat. Results are presented which indicate that certain combinations of simple edible chemicals (NaCl, $NaNO_3$, $NaNO_2$), in concentrations permitted by the FDA, effectively reduced the sterilizing dose of ground round beef, infected with massive numbers of Clostridium botulinum spores, by 0.5 to 1.0 Mrad.

The work covered by this report was performed by the Microbiology Department, Oregon State University, under Contract No. DA 19-129-QM-1517 during the period June 1962 to September 1963. A. W. Anderson was official investigator and P. R. Elliker, R. F. Cain, and K. L. Krabbenhoft were his collaborators.

The activities under this contract were monitored by the Microbiology Section, Q.M. Food & Container Institute*. Miss Dorothy A. Huber served as the Project Officer and Mr. Abe Anellis was Alternate Project Officer.

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## ABSTRACT

Spores of Clostridium botulinum (33A) were inoculated into canned ground round beef containing various additives and subsequently irradiated. Sodium nitrate (1000 ppm) plus sodium chloride (2.5%) completely inhibited spoilage for 120 days at 35°C when the samples were exposed to 2.0 Mrad, and no viable spores or toxin were recovered upon sub-culture. One hundred and twenty five cans were used per run. The use of either additive, with or without radiation, did not prevent spoilage unless higher levels of radiation such as 2.5 and 3.0 Mrad were used, with the concomitant production of undesirable odors.

Sodium nitrite (200 ppm) plus sodium chloride (2.5%) inhibited spoilage for 120 days but there was evidence of spore viability and toxin production in some of the unspoiled cans when the radiation level was 2.0 Mrad. Higher levels of radiation gave results similar to those obtained for sodium nitrate and sodium chloride.

## INTRODUCTION

Clostridium botulinum has been reported to be one of the most radiation resistant food spoilage bacteria (Morgan, 1953, 1954; Kempe, 1954). In addition C. botulinum produces a highly potent toxin and is a heat resistant, spore-forming organism which survives in many non-acid foods. For these latter reasons particularly, it is highly desirable that adequate means be developed to inhibit the growth of this organism in those foods where acid and/or heat cannot be used.

The use of radiation for the preservation of foods inoculated with various bacterial species has been reviewed by Niven (1958). Riemann (1962) has also recently reviewed the use of other agents to control the growth of C. botulinum. Among these, sodium nitrate, sodium nitrite and sodium chloride have been successfully applied to cheese, canned meat and other foods (Jensen, 1954; Silliker, 1958; Kempe, 1962). However, the complete inhibitory effect of sodium chloride and sodium nitrate at concentrations which meet Federal Food and Drug Administration specifications has not been substantiated by other workers (Hansen, 1955). It is also well known that when radiation is used as the sterilizing agent, it frequently causes serious defects in odor, flavor, color and texture in food. This is particularly true when high radiation levels are used, such as those required to destroy botulinal spores. Consequently it would be highly desirable to determine if certain chemical compounds in combination with radiation can effectively destroy or inhibit germination of botulinal spores; and equally important, to determine the minimal levels of radiation and test compounds which will consistently destroy these spores in food. A previous report on a preliminary study by Anderson (1962) indicates that when $NaNO_2$, $NaNO_3$ and NaCl are used in various combinations with irradiation (2.0 Mrad), there is inhibition of spoilage of canned ground round steak which has been inoculated with C. botulinum (type 5A) spores when incubated for 90 days or less at 35°C. However, viable spores were recovered in subculture and these produced a toxin lethal for mice. The present study was undertaken to determine which levels of certain test compounds and radiation will destroy botulinal spores when inoculated into canned ground beef. In addition, spores of type 33A will be used since recent studies (Anellis and Koch, 1962) have indicated that it is one of the most radiation resistant strains.

## MATERIALS AND METHODS

Preparation of Spores. The stock strain of C. botulinum, type 33A, was obtained from the Quartermaster Food and Container Institute for the Armed Forces, 1819 Pershing Road, Chicago, Illinois*. The spores were grown and harvested according to the procedure described below.

The inoculum was heat shocked for 10 minutes at 80°C. Immediately after heat shocking a 0.5% inoculum was introduced into 400 cc of freshly steamed trypticase thioglycollate broth (trypticase, 5.0%; Bacto-peptone, 1.5%; glucose, 0.01%; sodium thioglycollate, 0.01%). The inoculated flasks were then incubated for 6 days at 35°C in an anaerobic atmosphere (97% nitrogen and 3% carbon dioxide) to allow for the formation of a maximum number of spores.

After the 6 day growth period, the spore suspension was centrifuged (20 minutes at 2200 RPM). This was followed by six washings with sterile phosphate buffer (0.15M) adjusted to pH 7.0. Finally, the spores (titer $10^8$ spores/ml) were resuspended in the buffer and stored in screw cap tubes at 4-6°C.

For each experiment the refrigerated stock spore suspension was heat shocked and inoculated into 2500 cc of fresh trypticase-thioglycollate medium. After the 6 day growth period, the spores were centrifuged and washed (2 times in buffer) and resuspended in 200 ml of buffer. This latter suspension was used in making the viable spore count by the "most probably number" technique and was also the inoculum to be used in inoculating the meat prior to canning and irradiation. In all experiments the inoculum consisted of $1 \times 10^6$ spores per gram of ground round and was added just before the meat was canned.

Chemical Additives and Spore Inoculation. The following test compounds were used at the indicated levels in various combinations, with and without radiation:

| Test Compound | Levels |
|---|---|
| $NaNO_2$ | 100 & 200 ppm |
| $NaNO_3$ | 500 & 1000 ppm |
| NaCl | 1.5, 2.0 & 2.5% |

*Currently, Food Division, U. S. Army Natick Laboratories

The above substances were weighed out in flasks and sufficient water was added to make a heavy viscous suspension which was then added to the previously weighed meat sample (approx. 1000 gms). Following a thorough hand-mixing, the meat mixture was held at 4-6°C. for 18 hours to allow the added compound(s) to diffuse throughout the meat.

The meat was then inoculated with $1 \times 10^6$ viable, heat shocked spores per gram and thoroughly mixed. Approximately 100 gms. of the inoculated meat mixture was packed into baby food cans (202 x 204) and sealed. They were then packed in wet ice in an insulated ice chest and shipped to the Gamma Test Facilities, Arco, Idaho, for radiation. The cans were returned by the same method.

The meat used in these experiments was high-quality freshly ground round steak obtained from a locally operated government-inspected retail market.

Radiation Source. All cans in these experiments were irradiated at the Gamma Test Facilities, Arco, Idaho. The radiation levels used were 2.0, 2.5 and 3.0 Mrads.

Handling of Samples and Assay for Spore Survival. The irradiated samples returned to Oregon State University were incubated at 35°C. until a hard swell developed. They were then opened and examined microscopically to determine if spore growth had occurred. If no spoilage occurred within 120 days, the cans were opened and assayed.

The assay procedure consisted of opening the cans aseptically and removing a 10 gm. sample from various parts of the can with alcohol-flamed forceps. These samples were transferred to sterile screw cap tubes, covered with 7 ml. of steamed trypticase-thioglycollate broth and incubated anaerobically for 4 days at 35°C. The incubated subcultures were centrifuged at 2200 RPM for 20 minutes to separate the meat from the supernatant which contained the botulinal toxin. The supernatant was decanted off and divided into two aliquots. One portion was transferred to a sterile culture tube (15 x 150 mm) and served as the active preparation; the remaining portion was transferred into another culture tube and boiled for 15 minutes to inactivate any toxin present and to volatilize certain toxic substances such as ammonia. These latter tubes were again centrifuged and subsequently served as a control. Both sets of culture tubes were covered with rubber hooded stoppers and taped shut.

The liquid samples were then injected intraperitoneally into white mice (15-20 gms. each), using a dose of 0.20 ml. per mouse. Two mice received the unboiled inoculum and one received the boiled inoculum. Toxicity of the preparation was characterized by the typical symptoms of botulinal poisoning followed by death. Lethal effects were absent in the boiled control.

In order to ascertain the presence of viable spores in the toxin-containing samples, subcultures were made from the 10 gm. meat samples. These subcultures were then examined microscopically for presence of spores and toxin-producing ability.

## RESULTS AND DISCUSSION

Results shown in Table 1 indicate that C. botulinum spores (33A) are not destroyed when inoculated into canned ground round and irradiated (2.0 Mrad) in the presence of various combinations of $NaNO_2$ (100 ppm), $NaNO_3$ (500 ppm) and NaCl (1.5%). Microbial spoilage was retarded for 30-55 days when the irradiated cans contained $NaNO_3$ or $NaNO_3$ + NaCl. In all other cases, spore germination occurred as rapidly in the treated samples as in the controls. The uninoculated controls remained in satisfactory condition during the 120 day incubation period (35°C).

When $NaNO_2$ was used in higher concentrations (200 ppm) with irradiation, the rate and extent of spoilage was decreased but not prevented entirely (Table 2). The highest level of radiation used (3.0 Mrad) in combination with $NaNO_2$ gave 16% spoilage (4 out of 25 cans) whereas the lower levels of irradiation (2.0 and 2.5 Mrad) resulted in 50% spoilage (12 out of 25 cans). The use of $NaNO_2$ or irradiation separately was ineffective. Viable spores and lethal toxin were recovered from 7 out of 50 non-spoiled cans.

In Table 3 $NaNO_3$ was used at the 1000 ppm level. The extent of spoilage was reduced to 4% (1 can out of 25) and there were no recoverable viable spores or toxin when irradiated to 3.0 Mrad. However, the can contents possessed a very objectionable, rancid odor which may be attributed to the high radiation level used. Lower levels of irradiation resulted in 20-40% spoilage (out of 25 cans), but viable spores and lethal toxin were found in the unspoiled samples.

Since NaCl has long been used as a food preservative, this compound was tested at concentrations of 2 and 2.5% in combination with radiation doses which do not produce undesirable odors in the meat. The results of Table 4 indicate there was 60% spoilage (out of 30 cans) and a high rate (40-100%) of recovery of viable spores and lethal toxin from the unspoiled cans.

A previous report (Anderson, 1962) indicated that nitrates and nitrites in combination with sodium chloride prevented the development of C. botulinum spores (5A and 115B) in irradiated canned meat during a 90 day incubation period. It was felt desirable to test these substances on spores of C. botulinum (33A). This is reported to be one of the most radiation resistant strains of botulinum when irradiated in phosphate buffer (Anellis and Koch, 1962).

When $NaNO_2$ (200 ppm) plus NaCl (2.5%) were added to the inoculated canned meat and irradiated at 2.5 or 3.0 Mrad, no spoilage, recoverable viable spores, or lethal toxin (out of 60 cans, total) was found after 120 days of incubation. Using the same combination of chemicals and a lower level of radiation (2.0 Mrad) resulted in 0.8% spoilage (1 can out of 125, total) but 5 of the remaining 124 cans contained viable spores and toxin (Table 5). It is conceivable that the one spoiled can was due to mishandling at the radiation source or to a localized high spore concentration in the can and, hence, may not be significant. However, the observation that 4% of the unspoiled cans contained lethal toxin is of considerable importance in evaluating this treatment. Kempe (1962) inoculated high levels of C. botulinum (62A) into canned ground meat followed by irradiation and incubation for 6 months to 5 years. He found no visible spoilage or viable spores but found ample evidence of botulinal toxin. He attributed the presence of toxin to the original inoculum of heat shocked spores which, by themselves, contained sufficient toxin to kill mice. In the present experiment, viable spores were recovered from all samples which contained toxin even though there was no apparent spoilage. Hence, the use of $NaNO_2$ and NaCl in conjunction with radiation will prevent spoilage of inoculated meat, but there is evidence of spore viability even after 120 days of storage.

When $NaNO_3$ (1000 ppm) plus NaCl (2.0 & 2.5%) were incorporated into inoculated canned ground round and subsequently irradiated, no spoilage over the 120 day incubation period and no viable spores or toxin were found (Table 6).

All levels of radiation proved equally effective. This observation is perhaps more significant than some previous reports, since a large number of samples was analyzed (125 total at the 2.0 Mrad exposure level), and the incubation period was extended to 120 days (vs. 90 days). In addition, there was little, if any, undesirable odor from the meat when irradiated at the lowest level, and the color was quite comparable to that of fresh ground round.

There are several areas which merit further study. Do the growth temperature and media of the inoculum subsequently alter the radiation resistance of the spores when introduced into meat? It should also be emphasized that the inoculum consisted of $1 \times 10^6$ spores per gram of meat. Since Kempe (1962), Niven (1958) and Riemann (1962) reported that the inoculum size is an important factor in determining the required radiation sterilization dosage, it is possible that the levels of additives and radiation used in this experiment could be appreciably reduced if the inoculum size was decreased. It would seem more practical to devise a method of sterilization wherein the inoculum size more nearly approaches that found in naturally contaminated foods. Also, considerable basic research remains to be done in explaining the mechanism whereby nitrates, nitrites and sodium chloride function synergistically in preventing spore development in irradiated substances.

In conclusion it was observed that inoculated canned ground round was effectively sterilized by radiation when $NaNO_3$ and NaCl were used in combination as additives. The use of $NaNO_2$ plus NaCl was less effective, and nitrite, nitrate, sodium chloride and radiation when used separately, proved to be quite ineffective in preventing spoilage.

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TABLE 1

Spoilage of canned ground round beef inoculated with Clostridium botulinum (33A) plus test compound and irradiated at 2.0 megarad*

| Test Cmpd. | Conc. | C. botulinum Spores/gm. | Mrad. | Days of Incubation Before Spoilage | Per cent of Total Cans Spoiled** |
|---|---|---|---|---|---|
| None | -- | 0 | 0 | 5 | 100% |
| None | -- | $1 \times 10^6$ | 0 | 5 | 100% |
| $NaNO_2$ | 100 ppm | $1 \times 10^6$ | 0 | 5 | 100% |
| $NaNO_3$ | 500 ppm | $1 \times 10^6$ | 0 | 5 | 100% |
| $NaNO_2$ + NaCl | 100 ppm 1.5% | $1 \times 10^6$ | 0 | 5 | 100% |
| $NaNO_3$ + NaCl | 500 ppm 1.5% | $1 \times 10^6$ | 0 | 5 | 100% |
| None | -- | 0 | 2 | 120 | 0 |
| None | -- | $1 \times 10^6$ | 2 | 5 | 100% |
| $NaNO_2$ | 100 ppm | $1 \times 10^6$ | 2 | 5 | 100% |
| $NaNO_3$ | 500 ppm | $1 \times 10^6$ | 2 | 55 | 100% |
| $NaNO_2$ + NaCl | 100 ppm 1.5% | $1 \times 10^6$ | 2 | 15 | 100% |
| $NaNO_3$ + NaCl | 500 ppm 1.5% | $1 \times 10^6$ | 2 | 30 | 100% |

* Five cans were used per sample run

** Spoilage indicated by hard swell of the can and/or spoiled contents.

TABLE 2

Spoilage of canned ground round beef inoculated with *C. botulinum* (33A) plus $NaNO_2$ and irradiated at various dosage levels

| Test Cmpd. | *C. botulinum.* Conc. Spores/gm. | Mrad. | No. of cans | Days of incubation before spoilage | % Total cans spoiled | % Unspoiled cans containing mouse-lethal toxin* |
|---|---|---|---|---|---|---|
| $NaNO_2$ | 200 ppm 0 | 0 | 5 | 10 | 100% | --- |
| $NaNO_2$ | 200 ppm $1 \times 10^6$ | 0 | 25 | 15 | 100% | --- |
| None | --- $1 \times 10^6$ | 2 | 5 | 5 | 100% | --- |
| $NaNO_2$ | 200 ppm $1 \times 10^6$ | 2 | 25 | 120 | 48% | 0 |
| None | --- $1 \times 10^6$ | 2.5 | 5 | 120 | 80% | 0 |
| $NaNO_2$ | 200 ppm $1 \times 10^6$ | 2.5 | 25 | 120 | 52% | 25% |
| None | --- $1 \times 10^6$ | 3.0 | 5 | 120 | 40% | 0 |
| $NaNO_2$ | 200 ppm $1 \times 10^6$ | 3.0 | 25 | 120 | 16% | 14% |

* All cans which did not spoil after 120 days incubation (35°C) were checked for botulinum toxin. The presence of viable spores was indicated by microscopic examination of subcultures.

TABLE 3

Spoilage of canned ground round beef inoculated with *Clostridium botulinum* (33A) plus $NaNO_3$ and irradiated at various dosage levels

| Test Cmpd. | Conc. | *C. botulinum* Spores/gm. | Mrad. | No. of cans | Days of incubation before spoilage | % Total cans spoiled | % Unspoiled cans containing mouse-lethal toxin* |
|---|---|---|---|---|---|---|---|
| $NaNO_3$ | 1000 ppm | $1 \times 10^6$ | 0 | 25 | 5 | 100% | --- |
| $NaNO_3$ | 1000 ppm | 0 | 0 | 5 | 5 | 100% | --- |
| $NaNO_3$ | 1000 ppm | $1 \times 10^6$ | 2 | 25 | 120 | 20% | 10% |
| None | --- | $1 \times 10^6$ | 2 | 5 | 30 | 100% | --- |
| $NaNO_3$ | 1000 ppm | $1 \times 10^6$ | 2.5 | 25 | 120 | 40% | 20% |
| None | --- | $1 \times 10^6$ | 2.5 | 5 | 30 | 100% | --- |
| $NaNO_3$ | 1000 ppm | $1 \times 10^6$ | 3.0 | 25 | 120 | 4% | 0 |
| None | --- | $1 \times 10^6$ | 3.0 | 5 | 120 | 60% | 0 |

* All cans which did not spoil after 120 days incubation (35°C) were checked for botulinum toxin. The presence of viable spores was indicated by microscopic examination of subcultures.

TABLE 4

Spoilage of canned ground round beef inoculated with *Clostridium botulinum* (33A) plus NaCl and irradiated at 2.0 megarad

| Test Cmpd. | Conc. | *C. botulinum* Spores/gm. | Mrad. | No. of cans | Days of incubation before spoilage | % Total cans spoiled | % Unspoiled cans containing mouse-lethal toxin* |
|---|---|---|---|---|---|---|---|
| NaCl | 2.5% | 0 | 0 | 5 | 5 | 100% | --- |
| NaCl | 2.5% | $1 \times 10^6$ | 0 | 5 | 5 | 100% | --- |
| NaCl | 2.5% | $1 \times 10^6$ | 2 | 25 | 120 | 60% | 40% |
| NaCl | 2.0% | $1 \times 10^6$ | 2 | 5 | 120 | 60% | 100% |
| None | -- | $1 \times 10^6$ | 2 | 5 | 20 | 100% | --- |

* All cans which did not spoil after 120 days incubation (35°C) were checked for botulinum toxin. The presence of viable spores was indicated by microscopic examination of subcultures.

TABLE 5

Spoilage of canned ground round beef inoculated with Clostridium botulinum (33A) plus test compounds and irradiated at various dosage levels

| Test Cmpd. | Conc. | C. botulinum Spores/gm. | Mrad. | No. of cans | Days of incubation before spoilage | % Total cans spoiled | % Unspoiled cans containing mouse-lethal toxin* |
|---|---|---|---|---|---|---|---|
| $NaNO_2$ + NaCl | 200 ppm 2.5% | 0 | 0 | 5 | 25 | 100% | --- |
| $NaNO_2$ + NaCl | 200 ppm 2.5% | $1 \times 10^6$ | 0 | 25 | 80 | 100% | --- |
| None | --- | $1 \times 10^6$ | 2 | 5 | 7 | 100% | --- |
| $NaNO_2$ + NaCl | 200 ppm 2.5% | $1 \times 10^6$ | 2 | a. 25** b. 100*** tot. 125 | 120 | 0.8% | 4% |
| None | --- | $1 \times 10^6$ | 2.5 | 5 | 15 | 100% | --- |
| $NaNO_2$ + NaCl | 200 ppm 2.5% | $1 \times 10^6$ | 2.5 | a. 25** b. 10** tot. 35 | 120 | 0 | 0 |
| None | --- | $1 \times 10^6$ | 3.0 | 5 | 120 | 80% | 0 |
| $NaNO_2$ + NaCl | 200 ppm 2.5% | $1 \times 10^6$ | 3.0 | 25 | 120 | 0 | 0 |
| $NaNO_2$ + NaCl | 200 ppm 2% | $1 \times 10^6$ | 2.0 | 10 | 120 | 10% | 0 |

* All cans which did not spoil after 120 days were checked for botulinum toxin. The presence of viable spores was indicated by microscopic examination of subcultures.
** Results of preliminary study.
*** Results of final experiment.

TABLE 6

Spoilage of canned ground round beef inoculated with Clostridium botulinum (33A) plus test compounds and irradiated at various dosage levels

| Test Cmpd. | Conc. | C. botulinum. Spores/gm. | Mrad. | No. of cans | Days of incubation before spoilage | % Total cans spoiled | % Unspoiled cans containing mouse-lethal toxin* |
|---|---|---|---|---|---|---|---|
| $NaNO_3$ + NaCl | 1000 ppm 2.5% | 0 | 0 | 5 | 120 | 80% | 0 |
| $NaNO_3$ + NaCl | 1000 ppm 2.5% | $1 \times 10^6$ | 0 | 25 | 30 | 100% | --- |
| None | --- | $1 \times 10^6$ | 2 | 5 | 85 | 100% | --- |
| $NaNO_3$ + NaCl | 1000 ppm 2.5% | $1 \times 10^6$ | 2 | a. 25** b. 100*** tot. 125 | 120 | 0 | 0 |
| None | --- | $1 \times 10^6$ | 2.5 | 5 | 120 | 80% | 0 |
| $NaNO_3$ + NaCl | 1000 ppm 2.5% | $1 \times 10^6$ | 2.5 | 25 | 120 | 0 | 0 |
| None | --- | $1 \times 10^6$ | 3.0 | 5 | 120 | 0 | 0 |
| $NaNO_3$ + NaCl | 1000 ppm 2.5% | $1 \times 10^6$ | 3.0 | 25 | 120 | 0 | 0 |
| $NaNO_3$ + NaCl | 1000 ppm 2.0% | $1 \times 10^6$ | 2.0 | 10 | 120 | 0 | 0 |

* Those cans which did not spoil after 120 days were checked for botulinum toxin. The presence of viable spores was indicated by microscopic examination of subcultures.

** Results of preliminary study.

*** Results of final experiment.

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13 ABSTRACT

Spores of Clostridium botulinum (33A) were inoculated into canned ground beef containing various additives and subsequently irradiated. Sodium nitrate (1000 ppm) plus sodium chloride (2.5%) completely inhibited spoilage for 120 days at 35°C when samples were exposed to 2.0 Mrad, and no viable spores or toxin were recovered upon sub-culture. One hundred and twenty five cans were used per run. The use of either additive, with or without radiation, did not prevent spoilage unless higher levels of radiation such as 2.5 and 3.0 Mrad were used, with the concomitant production of undesirable odors.

Sodium nitrite (200 ppm) plus sodium chloride (2.5%) inhibited spoilage for 120 days but there was evidence of spore viability and toxin production in some of the unspoiled cans when the radiation level was 2.0 Mrad. Higher levels of radiation gave results similar to those obtained for sodium nitrate and sodium chloride.

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|---|---|---|---|---|---|---|
| | ROLE | WT | ROLE | WT | ROLE | WT |
| Inoculation | 8 | | | | 6 | |
| Clostridium botulinum | 1 | | 7 | | 6 | |
| Beef | 1,2 | | 7 | | 7 | |
| Canned | 0 | | 0 | | 0 | |
| Ground | 0 | | 0 | | 0 | |
| Round | 0 | | 0 | | 0 | |
| Irradiation | | | | | 6 | |
| Sodium nitrate | | | 6 | | 6 | |
| Sodium chloride | | | 6 | | 6 | |
| Sodium nitrite | | | 6 | | 6 | |
| Inhibition | | | 4 | | 7 | |
| Spores | 2 | | 4 | | 7 | |
| Viable | 0 | | 0 | | 0 | |
| Toxin | 2 | | 4 | | 7 | |
| Irradiated | | | 0 | | | |
| Non-irradiated | | | 0 | | | |

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